• Basic Research •

The expression of lacrimal androgen-binding proteins in mice *Pseudomonas aeruginosa* keratitis

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Received: 2019-03-04 Accepted: 2019-11-20

Abstract

● **AIM:** To investigate the expression of lacrimal androgen-binding proteins (ABPs) in mice *Pseudomonas aeruginosa* (*P. aeruginosa*) keratitis.

● **METHODS:** *P. aeruginosa* mice model from different gender was developed by intra-stromal injection. The expression of lacrimal ABPs in lacrimal gland specimens from *P. aeruginosa* keratitis mice was detected by the quantitative polymerase chain reaction (qRT-PCR). Corneal virulence was evaluated based on clinical scores. To study the mechanism of lacrimal ABPs' expression, experimental subjects were pre-treated with 4E-BP1 inhibitor, and were used to evaluate the expression levels by qRT-PCR.

● **RESULTS:** Compared with control groups, the expression of ABPα, ABPη and ABPζ in lacrimal gland from *P. aeruginosa* keratitis mice had no meaningful changes, while ABPε and ABPδ were significantly higher at 1d after infection. The expression of ABPδ in lacrimal gland of male mice was higher than female mice, regardless of whether or not *P. aeruginosa* keratitis occurred. After 4E-BP1 inhibitor subconjunctival injection or lacrimal injection, the expression of ABPδ and ABPε has no significant change compared with the control group.

● **CONCLUSION:** ABPδ and ABPε secreted by mice lacrimal gland may involve in the progress of alleviating the severity of corneal damage in *P. aeruginosa* keratitis. The expression of ABPδ and ABPε upon *P. aeruginosa* infection is independent of cap-dependent mRNA translation activated by 4E-BP1.

● **KEYWORDS:** keratitis; *Pseudomonas aeruginosa*; androgen-binding proteins; lacrimal gland

DOI:10.18240/ijo.2020.01.02

Citation: Lyu LY, Wang Q, Xu Q, Zhao WY, Yang H, Che CY. The expression of lacrimal androgen-binding proteins in mice *Pseudomonas aeruginosa* keratitis. *Int J Ophthalmol* 2020;13(1):7-10

INTRODUCTION

Bacteria are the leading cause of eye infections worldwide[1]. Ocular infections may damage the structures and lead to blindness and visual impairment without treatment[2]. *Pseudomonas aeruginosa* (*P. aeruginosa*), which was found in 50% of keratitis diagnoses[3], is the most frequent isolate of Gram-negative ocular infections[4]. *P. aeruginosa* was hard to eradicate efficiently due to acquired antibiotic resistance and pathoadaptation, making the urgent demands to seek for alternative therapeutic methods[5-7].

The tear fluid plays the key role in maintaining the stability of the intraocular environments by covering the anterior corneal surface. The discharge of tears can flush pollutants and irritants out, thereby playing the role as the first line of defense against the invasion of pathogens for the anterior eye[8-9].

The androgen-binding proteins (ABPs) containing a small family of secretory proteins were only found in mammalian lineage. High concentrations of ABPs were found in many mammalian secretions, such as fluids of the lacrimal gland, lung and salivary gland[10]. Five kinds of lacrimal ABPs are characteristic to mice，including ABPα (Scgb1b27), ABPζ (Scgb2b24), ABPη (Scgb1b2), ABPε (Scgb2b2) and ABPδ (Scgb2b20)[11]. Although the biological activities of ABPs in most individuals have not been fully characterized, it has been found that this family play an important role in the regulation of tissue repairment, inflammation, and tumorigenesis[12]. There is a slight self-healing tendency due to keratitis in mice, and lacrimal ABPs may play a role against bacterial keratitis.

Interestingly, the secretion of some lacrimal ABPs is sex-oriented. In the five lacrimal ABPs characteristic to mice, though ABPα and ABPζ are uncertain and ABPη and ABPε are unbiased, ABPδ shows obvious male bias[13]. Whether the gender response to *P. aeruginosa* keratitis is different is also an interesting topic.

Based on these, present studies were designed to investigate the expression levels and roles of lacrimal ABPs in *P. aeruginosa* keratitis with different genders, as well as part of the mechanism of ABPs' functions.

MATERIALS AND METHODS

Ethical Approval All treatments on mice were complied with the regulations of Statement on the Use of Animals in Ophthalmic and Vision Research announced by Association for Research in Vision and Ophthalmology (ARVO).

Anatomical Position of Lacrimal Gland The main lacrimal gland of mice is out of orbita, locating directly below the ear with the long axis perpendicular to the zygomatic arch and connecting to the eye surface through a long excretory duct[14].

The Establishment of Mouse *Pseudomonas aeruginosa* Keratitis Eight-week-old specific pathogen-free C57BL/6 mice (male and female) were purchased from the Changzhou Cavens Laboratory (Jiangsu Province, China). The standard *P. aeruginosa* strain was provided by the Affiliated Hospital of Qingdao University. Mice were anesthetized by chloral hydrate (0.08 mL/mouse) through intraperitoneal injection. One eye was randomly selected from each mouse. Next, a 33-gauge Hamilton syringe was inserted through the tunnel, and 2.5 μL bacterial suspension (2.5×10 bacteria/μL PBS) was injected into the corneal stroma[15]. The *P. aeruginosa*–infected mouse corneas exhibited stromal infiltration 1d post-infection. To investigate the expression of lacrimal ABPs in *P. aeruginosa* keratitis of the eye in mice, the mice were divided into four groups, including normal control female, normal control male, *P. aeruginosa* keratitis female and *P. aeruginosa* keratitis male. To know the mechanism of ABPs, the experimental eyes were received a subconjunctival injection (3 μL) containing 4E-BP1/eIF4E interaction inhibitor 4E1RCat (SelleckChem) or dimethyl sulfoxide (DMSO) as a control at 1d and 2h before infection in group 1. Same as above, the experimental lacrimal glands were received an injection (3 μL) containing 4E-BP1 inhibitor in group 2.

Clinical scores were used to evaluate the degree of corneal infections: 0, transparent or slight opacity, partly covering pupil; +1, slight opacity, completely covering cornea; +2, dense opacity, partly or completely covering pupil; +3, dense opacity, completely covering cornea; +4, corneal perforation or keratitis[16]. Lacrimal glands were collected one day after establishing the mouse model for quantitative polymerase chain reaction (qRT-PCR).

Quantitative Polymerase Chain Reaction Under an operating microscope, whole lacrimal gland of each mouse was then carefully cut off. RNA was extracted from mice lacrimal gland using RNAiso plus reagent (Takara). To obtain cDNA, the primescript RT Reagent Kit (Takara) was used to reverse transcript 2 μg total RNA. Using Eppendorf Mastercycler and SYBR green, qRT-PCR was performed when β-actin was used for internal control (Table 1).

Statistical Analysis Two-tailed, unpaired *t*-test was used to determine the statistical significance of qRT-PCR data and clinical score. Data were represented as mean±standard deviation and analyzed by GraphPad 7.0 software. When $P \leq 0.05$, differences were considered significant.

Table 1 Nucleotide sequences of mouse primers for qRT-PCR

Genes	Primer sequence (5'-3')
Scgb2b24-F	GGAAGCAGGCTGTGGTTGTATC
Scgb2b24-R	GGAATAGTACTGCAGGCATTCTGG
Scgb2b2-F	TCTCTGGAAACAGGATTGGGTTA
Scgb2b2-R	CGACCTGCATTCTGAGCTGAAG
Scgb2b20-F	GGTGTGGTTGTATCAAGAACTCCAG
Scgb2b20-R	AGACCATAGTATGACAGGCATTCAG
Scgb1b27-F	TCTGATAGGACCTTGACCGAGGA
Scgb1b27-R	GCTGCATCTATGCTGGTGAGGA
Scgb1b2-F	TCGATAGGACGTTGACGAAGG
Scgb1b2-R	GTAGGGCTTGTTGCATCTATGTAGG
β-actin-F	GATTAC TGCTCTGGCTCCTAGC
β-actin-R	GACTCATCGTACTCCTGCTTGC

RESULTS

The Establishment of *Pseudomonas aeruginosa* Keratitis Models in Female and Male Mice Images captured with a slit lamp after infection at 1d illustrated the disease response to different genders (Figure 1). Disease response was represented by a clinical score (n=8/group). There was no statistical difference between the two groups (P>0.05).

The Expression of Androgen-binding Proteins in *Pseudomonas aeruginosa* Keratitis Compared with normal groups, the expression of ABPα, ABPη and ABPζ in lacrimal gland had no meaningful changes (P>0.05), while ABPε and ABPδ were significantly higher (P<0.05) at 1d after infection (Figure 2). What's more, the expression of ABPδ in lacrimal gland of male mice was higher (P<0.05) than female mice, regardless of whether or not *P. aeruginosa* keratitis occurred.

The expression of ABPδ and ABPε upon *P. aeruginosa* infection was independent of cap-dependent mRNA translation activated by 4E-BP1. After 4E-BP1 inhibitor subconjunctival injection or lacrimal injection, the expression of ABPδ and ABPε had no significant change compared with the control group (P>0.05; Figure 3). There was also no significant difference between the two experimental groups (P>0.05).

After 4E-BP1 inhibitor subconjunctival injection or lacrimal injection, the expression of ABPδ and ABPε has no significant change compared with the control group (P>0.05). There was also no significant difference between the two experimental groups (P>0.05).

DISCUSSION

These results demonstrated that the expressions of ABPδ and ABPε were increased in lacrimal gland of both female and male mice against *P. aeruginosa* keratitis. As the members of lacrimal ABPs, ABPδ and ABPε may be involved in *P.*

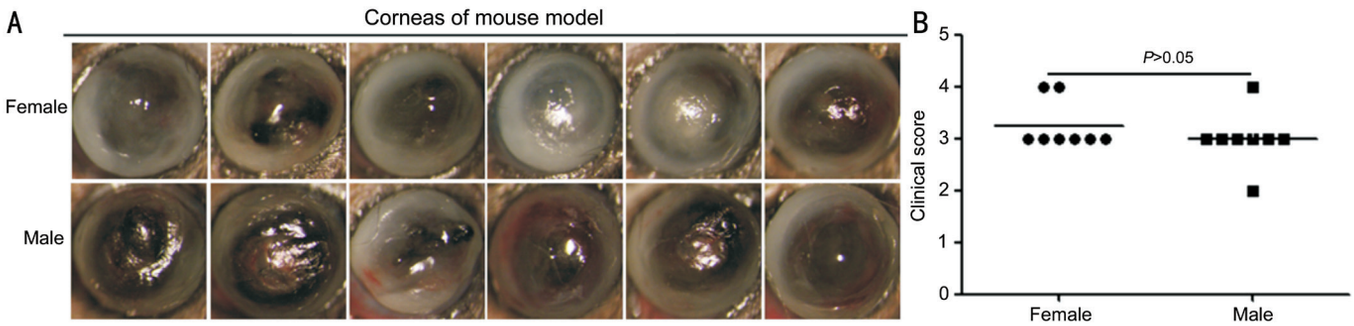

Figure 1 The establishment of a *P. aeruginosa* keratitis model in female and male mice A: Images captured with a slit lamp at 1d after infection; B: Disease response was showed by a clinical score (n=8/group), which was no statistical difference between the two groups (P>0.05).

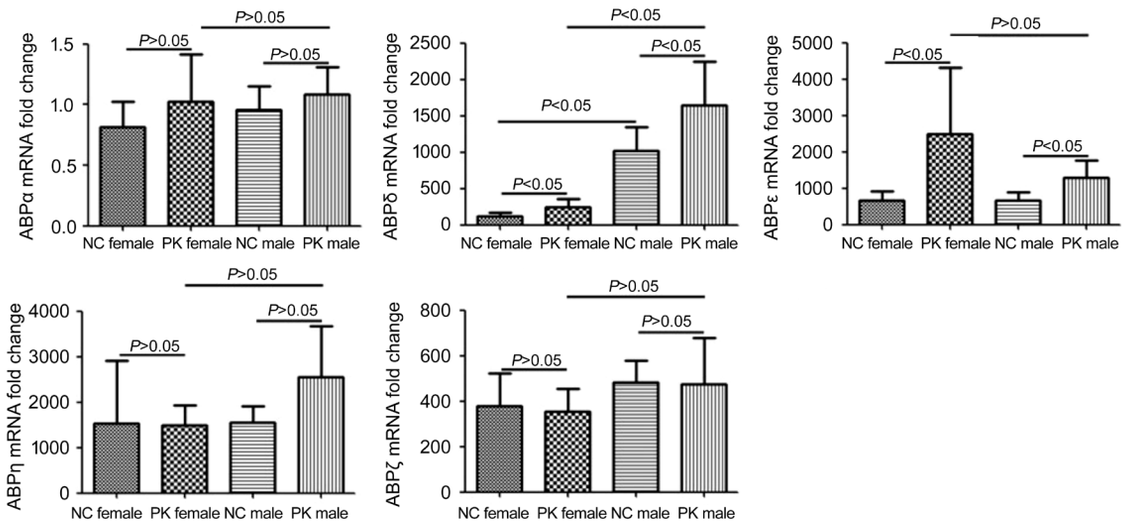

Figure 2 The expression of lacrimal ABPs in mice *P. aeruginosa* keratitis The expression of ABPα, ABPη and ABPζ in lacrimal gland had no meaningful changes (P>0.05), and the expression of ABPε and ABPδ were significantly higher (P<0.05) at 1d after infection. Meanwhile, the expression of ABPδ in lacrimal gland of male mice was higher (P<0.05) than female mice, regardless of whether or not *P. aeruginosa* keratitis occurred.

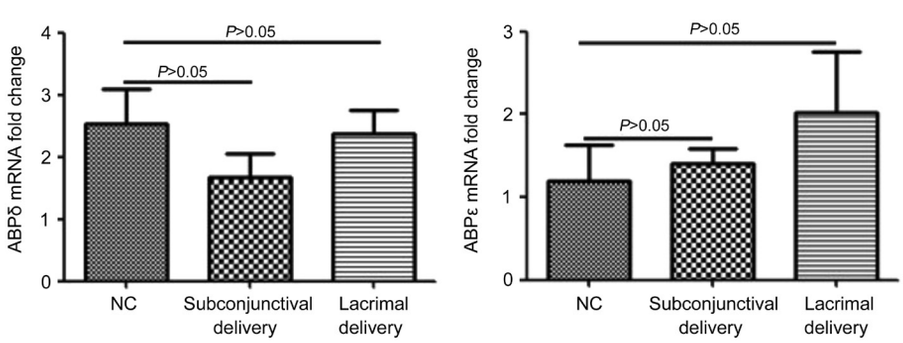

Figure 3 The expression of ABPδ and ABPε upon *P. aeruginosa* infection was independent of cap-dependent mRNA translation activated by 4E-BP1.

aeruginosa inflammation. Though the character of ABPs in *P. aeruginosa* keratitis remains unclear, number of researches have shown their anti-inflammatory effect. For instance, under the stimulation by the ABP dendrimer, interleukin (IL)-10 could be produced by the same cellular subsets *in vitro* among human immune cells[15,17]. And the production of IL-10 was known as the paradigm of anti-inflammatory cytokines[11]. Taken together, lacrimal ABPδ and ABPε may have a protective effect in *P. aeruginosa* keratitis.

In addition, the expression of ABPδ in lacrimal gland of male mice was higher than female mice, regardless of whether or not *P. aeruginosa* keratitis occurred in our study. This showed a clear gender bias. There was no significant difference between male and female mice in *P. aeruginosa* keratitis. Besides, the expression of ABP ε was slightly higher in female mice although without statistical difference. The expression of ABP ε may be a kind of compensation for the lower expression of ABP δ in female mice.

Eukaryotic translation initiation factor (eIF4E), as the eukaryotic initiation factor, regulates the association between eIF4F and the mRNA 5′-cap structure to stimulate the initiation of cap-dependent translation in the cytoplasm. It's an essential effector of cellular survival and proliferation under most circumstances. Two major pathways are used by the eIF4E to regulate the expression of genes related to proliferation, apoptosis, and cell growth: mRNA export and cap dependent translation[18]. 4E-BP1 can prevent the recruitment of other translation factors and down-regulate translation by blocking the interaction with eIF4G in space[19-20].

After 4E-BP1 inhibitor subconjunctival injection or lacrimal injection, the expression of ABPδ and ABPε had no significant change compared with the control group. The expression of ABPδ and ABPε upon *P. aeruginosa* infection was independent of cap-dependent mRNA translation activated by 4E-BP1.

In summary, ABPδ and ABPε secreted by mice lacrimal gland may be involved in the progress of alleviating the severity of corneal damage in *P. aeruginosa* keratitis. The expression of ABPδ and ABPε upon *P. aeruginosa* infection was independent of cap-dependent mRNA translation activated by 4E-BP1. Unfortunately, the researches on lacrimal ABPs are still rare. Moreover, there is no commercial antibody to ABPs currently. So we bring the preliminary results about ABPs induced by *P. aeruginosa* keratitis in this study. It's promising that the veil of ABPs will eventually be lifted in the further researches on ABPs.

ACKNOWLEDGEMENTS

Foundations: Supported by the National Natural Science Foundation of China (No.81300730); China Postdoctoral Science Foundation (No.2018M630482); Key Research Project of Shandong Province (No.2018GSF118193); Science and Technology Project of Qingdao (No.19-6-1-39-nsh); Qingdao Outstanding Health Professional Development Fund.

Conflicts of Interest: Lyu LY, None; **Wang Q,** None; **Xu Q,** None; **Zhao WY,** None; **Yang H,** None; **Che CY,** None.

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